



# 2x Blue qPCR Probe Master Mix - high ROX

| Article         | Content                     |
|-----------------|-----------------------------|
| SL-9802HRB-smp  | 1 ml, 100 rxn × 20 µl       |
| SL-9802HRB-5ML  | 5 x 1 ml, 500 rxn × 20 µl   |
| SL-9802HRB-10ML | 10 x 1 ml, 1000 rxn × 20 µl |
| SL-9802HRB-20ML | 20 x 1 ml, 2000 rxn × 20 µl |



Long-Term Storage at -20°C in the dark

Short-Term Storage at 4°C in the dark

#### DESCRIPTION

Our **primaQUANT PROBE Blue 2x qPCR Master Mix** is an optimized ready-touse mixture for probe-based assays such as Taqman®, Beacons and MGBs. It contains a modified HotStart DNA Polymerase, as well as dNTPS and MgCl<sub>2</sub>. The sophisticated buffer system provides fast kinetics and target amplification even for difficult templates.

The **primaQUANT 2x Master Mix** contains all components - you just need to add primers and template DNA/cDNA. The **blue color does not interfere** with the qPCR reaction but helps you to track wells already filled during pipetting steps.

### **DID YOU KNOW?**

- primaQUANT PROBE is also offered as a blue-colored mix for better handling.
- This Master Mix contains high ROX at a final concentration of 500 nM.
- For qPCR cyclers requiring other concentrations of ROX, primaQUANT PROBE is also available with high concentrations of ROX or without ROX.



# **Recommended Reaction Mixture per Well**

### **BEFORE YOU START**

- After thawing, please invert the Master Mix tube 6-8 times.
- **Do not vortex** the Master Mix to avoid damaging the enzyme.

| Component                   | Stock<br>Concentration | 20 µl<br>Reaction  | 10 µl<br>Reaction  | Final<br>Concentration               |
|-----------------------------|------------------------|--------------------|--------------------|--------------------------------------|
| 2x primaQUANT<br>Master Mix | 2x                     | 10 µl              | 5 µl               | 1x                                   |
| Forward Primer              | 4 µM                   | 1µl                | 0.5 µl             | 200 nM (100 - 400 nM<br>recommended) |
| Reverse Primer              | 4 µM                   | 1µl                | 0.5 µl             | 200 nM (100 - 400 nM<br>recommended) |
| Probe                       | 8 µM                   | 1µl                | 0.5 µl             | 400 nM (200 - 600 nM<br>recommended) |
| Template DNA                | -                      | variable           | variable           | 0.1 - 10 ng per reaction             |
| Sterile Water               | -                      | adjust<br>to 20 µl | adjust<br>to 10 µl | -                                    |



### -<u>()</u>- NOTE

For maximum efficiency and specificity, adjustments of annealing temperature as well as extension time, primer/probe concentration and template concentration may be needed.

### CALCULATOR TOOL

Please feel free to download our Excel sheet calculator to calculate the necessary volumes: calculator.steinbrenner-laborsysteme.de.





# **Standard Protocol**



- For the majority of qPCR assays, standard cycling conditions can be applied for the majority of qPCR assays out-of-the box.
- However, cycling conditions strongly depend on the primer, probe, amplicon and input material and thus some of these factors might need adjustments.

#### **3-STEP PROTOCOL**

| Step                 | Time            | Temperature                 |                |
|----------------------|-----------------|-----------------------------|----------------|
| Initial Denaturation | 1 - 3 minutes   | 92°C - 95°C                 |                |
| Denaturation         | 5 - 10 seconds  | 92°C - 95°C                 |                |
| Annealing            | 1 - 5 seconds   | 60°C<br>depending on primer | 25 - 40 cycles |
| Extension            | 10 - 20 seconds | 72°C                        |                |

### **2-STEP PROTOCOL**

| Step                              | Time            | Temperature                 |                |
|-----------------------------------|-----------------|-----------------------------|----------------|
| Initial Denaturation              | 1 - 3 minutes   | 92°C - 95°C                 |                |
| Denaturation                      | 5 - 10 seconds  | 92°C - 95°C                 |                |
| Annealing /<br>Extension combined | 10 - 20 seconds | 60°C<br>depending on primer | 25 - 40 cycles |



# **Ultra-fast Protocol**



- Ultra-fast cycling conditions highly depend on the ramping rate of your qPCR cycler, primer, probe, amplicon and input material and thus might need adjustments.
- Ultra-fast cycling conditions can be applied for the majority of qPCR assays out-of-the box, provided that your primer/probe sets do not show unspecific binding.

#### **3-STEP PROTOCOL**

| Step                 | Time          | Temperature                 |                |  |
|----------------------|---------------|-----------------------------|----------------|--|
| Initial Denaturation | 1 minute      | 92°C - 95°C                 | L CEE          |  |
| Denaturation         | 1 - 5 seconds | 92°C - 95°C                 |                |  |
| Annealing            | 1 - 5 seconds | 60°C<br>depending on primer | 25 - 40 cycles |  |
| Extension            | 1 second      | 72°C                        |                |  |

### **2-STEP PROTOCOL**

| Step                              | Time          | Temperature                 |                |
|-----------------------------------|---------------|-----------------------------|----------------|
| Initial Denaturation              | 1 minute      | 92°C - 95°C                 |                |
| Denaturation                      | 1 second      | 92°C - 95°C                 |                |
| Annealing /<br>Extension combined | 1 - 5 seconds | 60°C<br>depending on primer | 25 - 40 cycles |

### MANUAL



### S primaQUANT PROBE

# **Applications**

Probe-based quantitative PCR

- TaqMan® Probes
- Any kind of Dual-Labeled Hydrolysis Probe
- Molecular Beacons
- Scorpion Probes

DNA Genotyping DNA SNP Analysis RNA and miRNA Expression Multiplexing (up to 4 colors) Transcript Variant Analysis

### QUALITY CONTROL PROCEDURE

Our **primaQUANT PROBE 2x qPCR Master Mix** undergoes stringent quality controls. Each lot is tested in a probe-based qPCR with cDNA and lambda DNA input.

Enzyme purity and homogenity of > 98 % is validated using a Bioanalyzer SDS protein electrophoresis.

All **primaQuant** Master Mixes are free of detectable endonuclease- & exonuclease activity:

- Incubation of 1 µg of plasmid DNA with 5 U for 4h at 37°C and 72°C
- Incubation of 1 μg of a DNA size standard with 5 U for 4h at 37°C and 72°C

For more information, please visit our website: www.steinbrenner.de



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# **Further Products**

Products that may also interest you

#### **REVERSE TRANSCRIPTION KIT**

S primaREVERSE RT-KT

For the efficient cDNA synthesis out of total RNA extractions try the primaREVERSE RT-Kit with article number SL-9540.